

Fast Methods for Small Molecules

Technical Overview

Throughput is a key concern in many NMR laboratories, and using faster methods is one way to increase it. Traditionally, multi-dimensional NMR requires stepwise increments of a delay in the pulse sequence to sample an entire range of frequencies, which can consume a lot of time, as the number of increments could be in the hundreds. By exciting and sampling only the frequencies of interest in the indirect dimension (F1), the data acquisition time can be significantly reduced. This can be accomplished using Hadamard spectroscopy. This Technical Overview illustrates the unique ability of the VnmrJ 3.1 software to increase throughput by enabling easy setup of Hadamard experiments (state of the art fast methods) for small molecules.



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Hadamard spectroscopy

Hadamard spectroscopy is based on the selective excitation of a predetermined set of frequencies, which replaces the traditional non-selective RF pulse(s) and the evolution delay for spin excitation and frequency encoding (Figure 1). A Hadamard matrix is used

to determine the order of modulation, excitation or inversion, of each signal (encode). The advantage of using a Hadamard matrix over a sequential approach of selectively exciting a single signal of interest (i.e., several selective 1D experiments, one per frequency of interest) is the additive effect each row has when decoding the data, providing the same multiplex advantage as

standard time domain NMR spectroscopy^{1,2}. The Hadamard matrix used for frequency encoding is then used at the automated processing stage to decode the data acquired, which is then Fourier transformed to produce the final 2D spectrum. Provided sensitivity is sufficient, a 2D Hadamard experiment can be acquired in less than 1 minute.

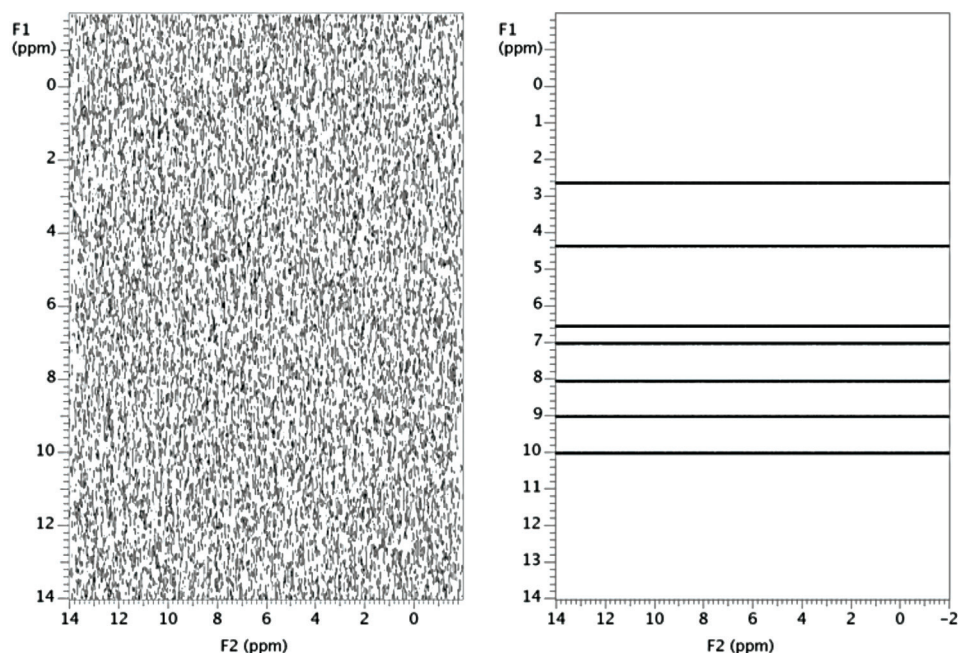


Figure 1. Two Fourier transformed 2D ^1H - ^1H COSY noise-only spectra, acquired with 128 stepwise increments to sample a 16 ppm range (left), and with only 7 frequencies of interest excited (right). Using only the 7 frequencies, horizontal lines are present and there is no noise in any other region of the spectrum.

Hadamard Experiments in VnmrJ 3.1

Many experiments are available with Hadamard encoding in the VnmrJ 3.1 software (Table 1), and they can be used either in a manual mode (i.e., running one experiment at a time in foreground with individual optimization) or in an automated mode through the Study Queue with the same flexibility for optimization of individual experiments. The data is as easy to interpret

as the equivalent non-Hadamard experiments. While generation of the Hadamard matrix requires foreknowledge of resonance positions, the methodology is very beneficial, particularly for ^1H or ^{19}F homonuclear 2D experiments, because ^1H or ^{19}F frequencies can be gotten from a quick 1D spectrum. The experiments with the most obvious advantage are the ROESY and NOESY experiments, as they typically require many repetitions per increment.

Table 1. Experiments with Hadamard Encoding in the VnmrJ 3.1 Software

Homonuclear experiments	Heteronuclear Experiments
Correlation Spectroscopy (COSY)	Heteronuclear Single Quantum Coherence (HSQC)
Double Quantum filtered Correlation Spectroscopy (DQCOSY)	Heteronuclear Multiple Bond Correlation (HMBC)
Total Correlation Spectroscopy (TOCSY)	Heteronuclear Single Quantum Coherence Total Correlation Spectroscopy (HSQCTOCSY)
Nuclear Overhauser Effect Spectroscopy (NOESY)	Heteronuclear Correlation (HETCOR)
Rotating frame Overhauser Enhancement Spectroscopy (ROESY)	

Setting up Hadamard experiments

The setup of Hadamard experiments is easy, using very similar workflows to traditional experiments. They can be run interactively in the foreground with full control by the investigator, or in automation. Setup within the Study Queue starts with the acquisition of a 1D spectrum which is used to generate a list of frequencies of interest. The Edit Hadamard Encoding Frequencies

dialog box shown in Figure 2, which is accessible under the Experiments pull down menu, displays frequencies in the Line List table automatically as they are selected from the spectrum. The frequencies can also be entered manually or imported from traditional threshold-based line listing routines. Entries are either frequencies (Hz) from transmitter offset or chemical shifts (ppm).

The line lists are then stored with their respective 1D spectra, to be called automatically when needed.

The Hadamard experiments from the Experiment Selector can be added to the Study Queue and are treated like any other experiment running in the Study Queue. Further customization of each experiment can be done by the investigator (as in the case of setting these experiments in foreground).

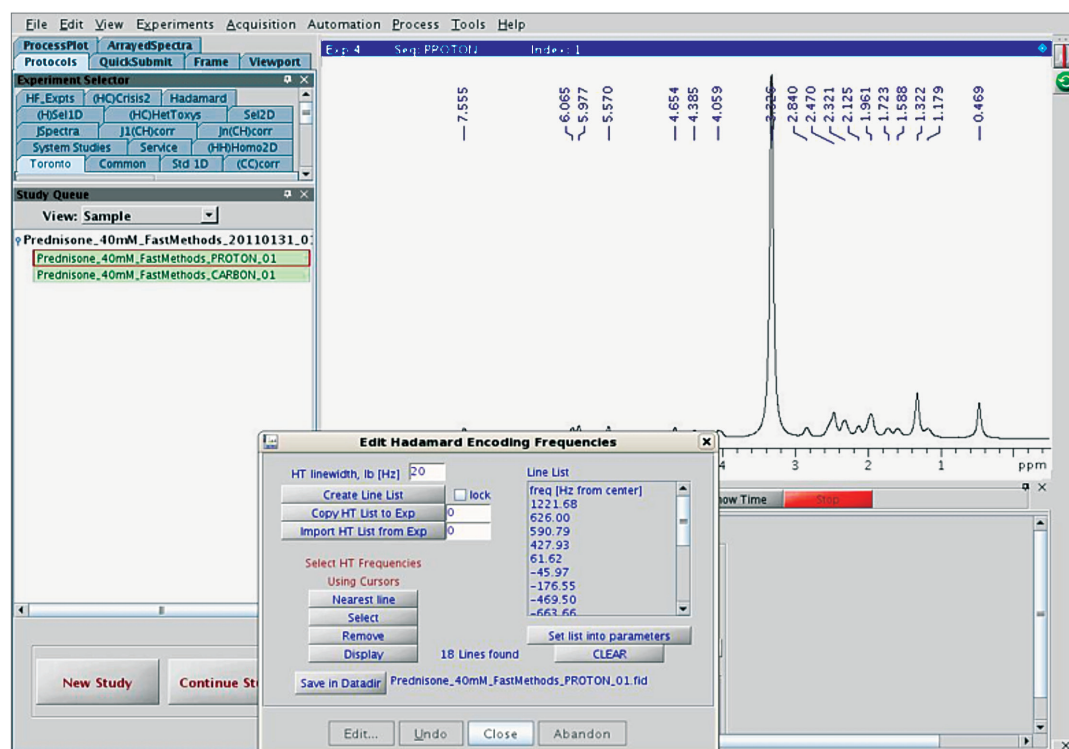


Figure 2. The generation of a list of peaks to be used for ^1H detected Hadamard experiments. The high resolution ^1H spectrum is processed with 20 Hz line broadening to reduce the possibility of picking peaks of the same ^1H . The list is saved in the parameter set of the 1D experiment and automatically recalled before the Hadamard experiment is run in the Study Queue. A list of 18 peaks is depicted, which was created for a 40 mM sample of Prednisone acquired on a 400 MHz system with a OneNMR Probe and ProTune PZT installed.

Figure 3 depicts a Hadamard COSY experiment being customized and set to run for approximately 100 seconds. There are 18 peaks in the Proton spectrum which VnmrJ 3.1 automatically assigns a Hadamard matrix size of 32. The resolution in F1 of each entry on the line list is defined by bandwidth selected; here all frequencies are set

a to 20 Hz bandwidth. A traditional COSY with the same resolution as this Hadamard equivalent would require 200 increments. In terms of total experiment time, this would be nearly 11.25 minutes for the traditional COSY compared to 100 seconds for the Hadamard COSY.

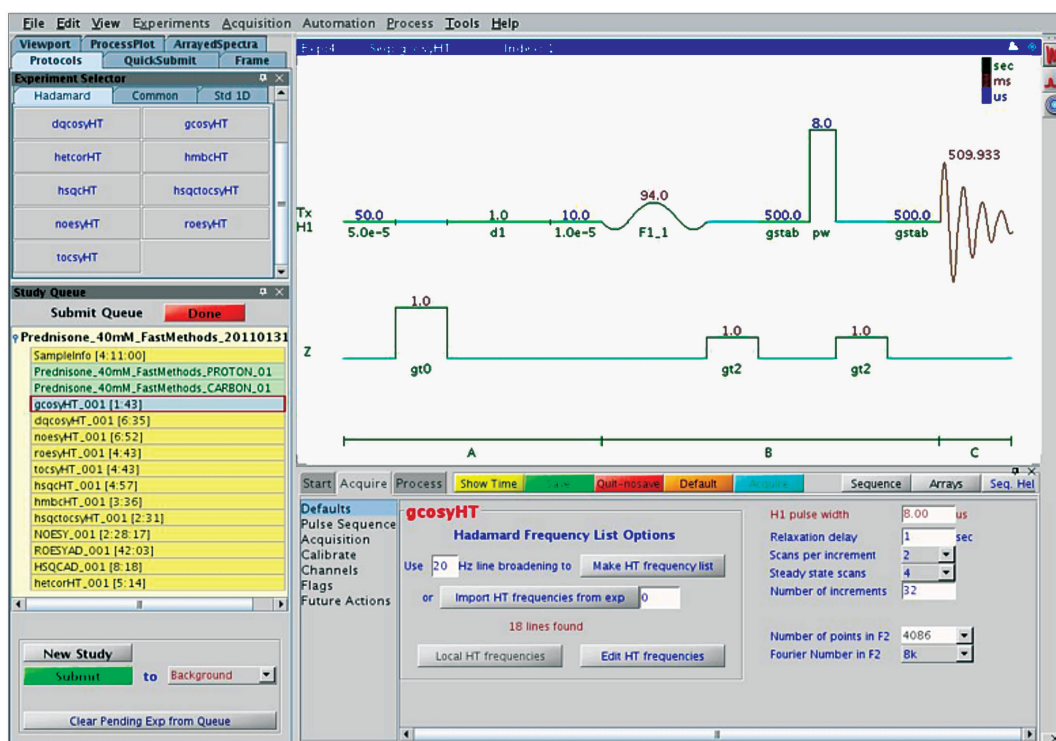


Figure 3. Hadamard experiments queued up to run automatically in the Study Queue. The COSY experiment has been loaded and the default customization panel shown, with 32 increments for a Hadamard matrix of $N=2^5$. The direct dimension (F2) is set for 4086 data points. The total experiment time was 100 seconds.

Examples of Hadamard experiments

Two homonuclear Hadamard experiments, shown in Figures 4 and 5 along with their linear stepwise counterparts, demonstrate that the data obtained are comparable while time saving is about an order of magnitude.

Processing times are faster than traditional Fourier transform of the non-Hadamard experiments, and the plotting of Hadamard experiments is seamlessly integrated, just like it is for non-Hadamard experiments. Using the Study Queue, Hadamard experiments can also be scheduled to run

at a later time, facilitating much more efficient use of spectrometer time. This feature enables the use of multi-dimensional techniques (e.g., NOESY, HSQC) on more samples and thus higher throughput rates by running the faster Hadamard experiments at off-hours.

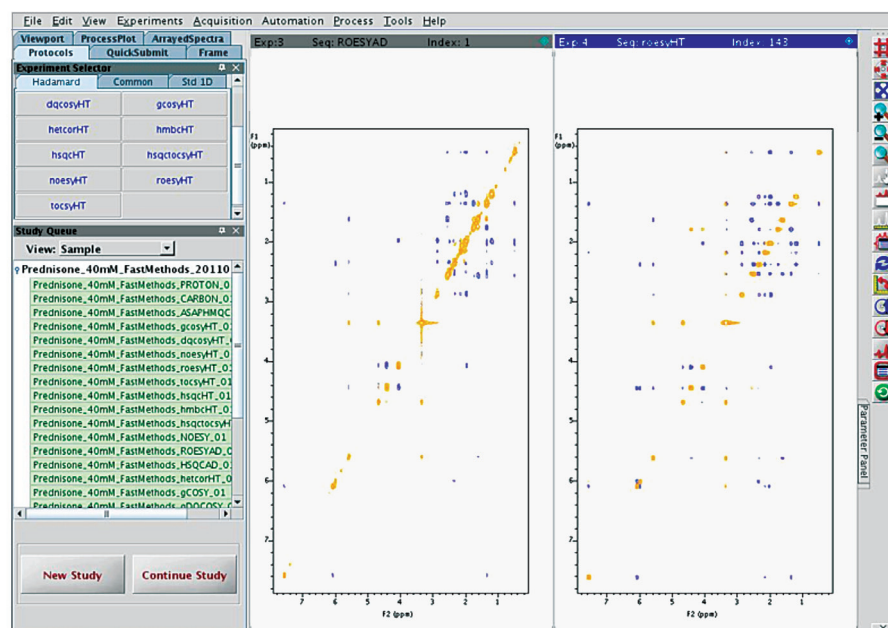


Figure 4. A linear stepwise ROESY experiment of 200 increments (left) which took ~ 40 minutes compared to about 4 minutes with a Hadamard encoded ROESY experiment (right).

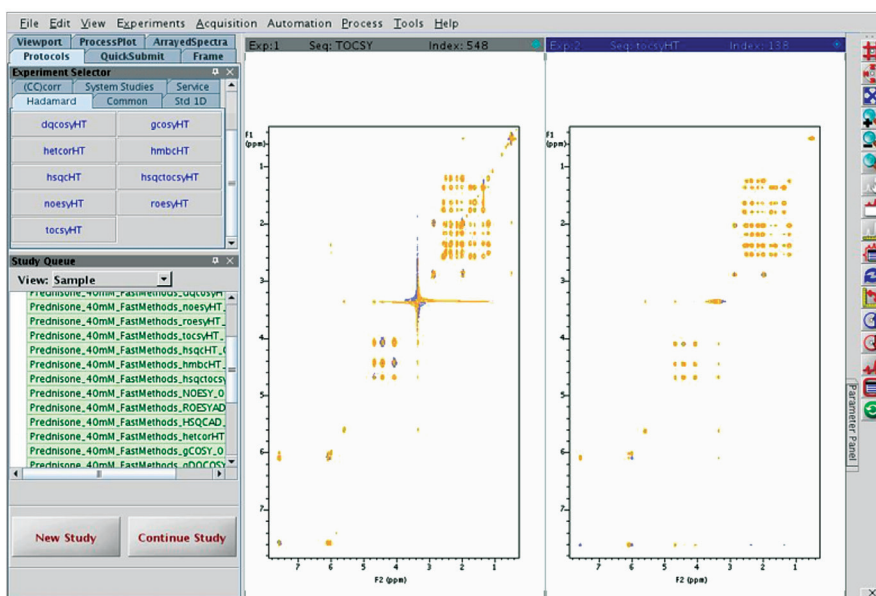


Figure 5. A linear stepwise TOCSY experiment of 200 increments (left) which took about 67 minutes, compared to about a 5 minute Hadamard encoded TOCSY experiment (right).

The Hadamard HSQC experiment displayed in Figure 6 shows a hetero-nuclear experiment that was acquired under automation, in which the 1D ^{13}C spectrum was used to generate the line list for the experiment. This experiment used 32 increments, and the resolution in F1 of each entry on the line list was defined by bandwidth selected, again set to 20 Hz bandwidth. The equivalent traditional HSQC experiment, with 96 increments and a spectral width of 20.1 kHz, would have a resolution of only 208 Hz, which corresponds to 2.1 ppm on a 400 MHz system. To achieve the same resolution as the Hadamard equivalent, this traditional HSQC experiment would require 1024 increments, and the experimental time would be nearly 5 hrs and 14 minutes, compared to 5 minutes.

Conclusions

Hadamard spectroscopy offers a speed advantage in multi-dimensional NMR over linear, stepwise sampling. The time savings can be orders of magnitude, as the Hadamard matrix excludes "empty" spectral regions which linear sampling does not. Hadamard spectroscopy takes advantage of excellent RF and digital hardware, which provides the precise execution of RF events required for the addition and subtraction of data. The VnmrJ 3.1 software enables easy setup and running of Hadamard experiments by calculating, creating and implementing multi-frequency shaped pulses automatically. These Hadamard experiments can be run automatically (in background, foreground or using a sample

changer) from within the Study Queue. The processing, which is a matter of adding and subtracting the resultant raw data to recover individual sub-spectra, is fully integrated and seamless for the users. Hadamard spectroscopy of small molecules is easy to use in the VnmrJ 3.1 software for a wide range of fast homo-nuclear and heteronuclear experiments, and it can significantly improve the throughput of an NMR laboratory.

References

1. E. Kupce, T. Nishida, R. Freeman, "Hadamard Spectroscopy." *Progress in NMR Spectroscopy* **42**, 95-122 (2003).
2. E. Kupce, and R. J. Freeman, "Two-dimensional Hadamard spectroscopy" *J. Magn. Reson.* **162**, 300-310 (2003).

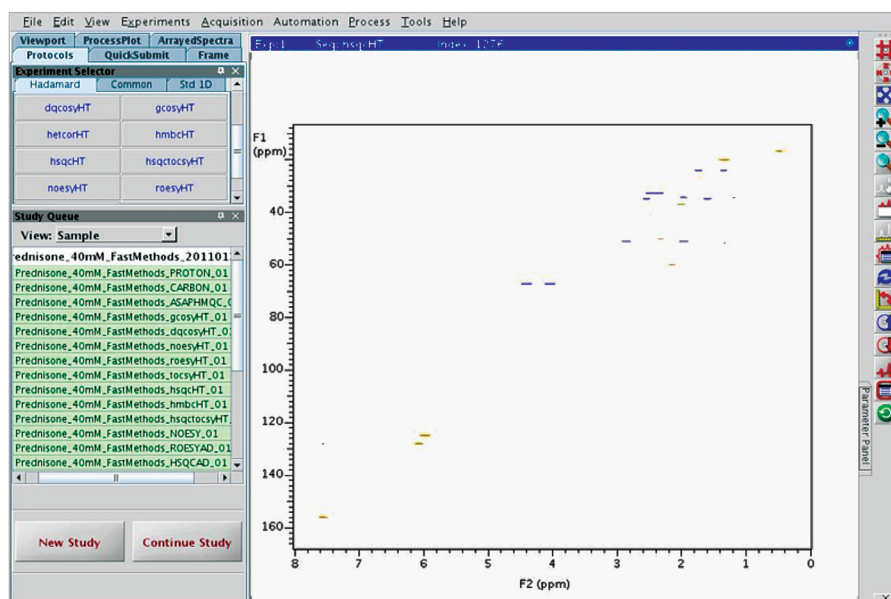


Figure 6. A 5 minute multiplicity-edited Hadamard HSQC on a 40 mM sample of Prednisone acquired through the Study Queue. Methyls and methines are colored differently than methylenes.

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